Introduction:

Exposure to altitude has specific biological effects in human. The physiological adjustments to hypoxia occur at systemic and cellular levels. Hypoxia elicits specific molecular responses in skeletal muscles to adapt to altered demands (Hoppeler and Vogt, 2001). Oxygen transport capacity can be improved on continuous residence at moderate heights (2,000-2,500m) by an erythropoietin-induced increase in the hematocrit (Bunn and Poyton, 1996). An increase in the haemoglobin concentration has been shown to enhance maximal O_2 consumption (VO_2max) and thus exercise performance (Ferretti et al., 1992). Athletes seek to boost their endurance or physical performance by training under hypoxic/high altitude conditions (Saunders et al., 2009). It leads to increased capillarisation, increased mitochondrial density and enhanced performance. Furthermore, training under normobaric/intermittent hypoxia has shown its potential in enhancing physical performance (Zoll et al., 2006;Bonetti and Hopkins, 2009).

The main molecular mechanism behind hypoxia training is a transcription factor called Hypoxia Inducible factor (HIF-1) which is known to master regulate several genes that are primarily responsible for systemic and muscular adaptation to hypoxia by enhancing physiological attributes like erythropoiesis, angiogenesis, glucose uptake and metabolism (Wenger, 2002). It is a dimeric protein composed of the regulatory subunit HIF-1α, which is encoded by 15 exons and the constitutively expressed subunit HIF-1β, which is identical to the aryl hydrocarbon receptor nuclear translocator (ARNT). It is the member of PAS domain containing protein family. Both HIF-1α and HIF-1β are constitutively expressed in cells but HIF-1α degrades readily under normoxic conditions via a proteasome dependent pathway. In the presence of oxygen and Fe^{2+}, prolyl hydroxylase (PHDs) enzymes hydroxylate two specific sites of HIF-1α. A common feature of this class of enzymes is the absolute requirement for Fe^{2+} and the cosubstrates 2-oxoglutarate and O_2 (Nagel et al., 2010). In a two-step reaction, 2-oxoglutarate is first decarboxylated to succinate and a reactive iron-oxo complex is formed that subsequently hydroxylates the defined amino acid residue of the peptide substrate. After hydroxylation, HIF-1α is ubiquitinylated by pVHL (Von Hippel Lindau protein) and it is degraded by proteasomes.

Under hypoxic conditions, the hydroxylation of HIF-1α does not take place and it escapes degradation. It is then transported to the nucleus and combines with HIF-1β to make dimer (HIF). Transactivation of HIF-1α is necessary for the induction of several genes, such as those encoding the glycolytic enzymes, glucose transporters (GLUT-1, GLUT4), the vascular endothelial growth factor (VEGF) as well as other metabolic proteins (Wenger and Gassmann, 1997). HIF-1 is expressed in all mammalian tissues, including skeletal muscle (Wiener et al., 1996). In skeletal muscles, these physiological adjustments lead to increase in oxygen delivery and metabolite utilization that result in enhanced performance (Hoppeler and Vogt, 2001;Zoll et al., 2006). Despite all benefits, the practical application of altitude training is contradictory because of ailments like acute mountain sickness which influence the efficacy of altitude training. Furthermore, hypoxia for a longer period causes deleterious effects in human being viz. tissue damage, edema etc that are potentially fatal conditions. Various other alternative methods are also available for providing simulated hypoxia

condition at sea levels viz. nitrogen houses, hypobaric chambers, altitude tents or hypobaric inhalers; although these can reduce the problem of high altitude illness to an extent but all of these methods have some limitations like high costs and limitations of number of people that can be acclimatized at a definite time period. Preconditioning can be defined as a process which eventually leads a tissue to be more tolerant to any lethal insult like hypoxia or ischemia where availability of oxygen is hampered. Chemical preconditioning has advantages over other types of preconditioning as it reduces acclimatization schedule at altitude thus saves time, a large group of people can be preconditioned simultaneously, so number is not a limitation as compared to simulation chambers. It is cost effective & easy to implement and thus economical.

Several studies have found that PHD inhibitors can reiterate various cellular responses to hypoxia or preconditioning stimuli. These include HIF-1α stabilization, induction of hypoxia inducible genes, stimulation of angiogenesis and protection against metabolic stress (Warnecke et al., 2003;Wright et al., 2003;Asikainen et al., 2005). PHD inhibitors include 2-Oxoglutarate analogues. This is the most important class and recently many of these kinds of molecules are in focus. These includes N-oxalylglycine, 3,4-dihydroxybenzoate (3,4-DHB) and L-Mimosine (L-Mim). Protocatechuic acid (3, 4-dihydroxybenzoic acid) is a phenolic compound found in many plant foods such as olives, Hibiscus sabdariffa (roselle), Eucommia ulmoides (du-zhong), and white grape wine is a 2- Oxoglutarate analogues. So far, less information is available regarding the content of this compound in fresh fruits. Its ester derivative ethyl 3, 4-dihydroxybenzoate (EDHB), a white crystalline powder is more permeable to cell and is widely used as a food additive. Ethyl 3, 4, dihydroxybenzoate is known to possess antioxidant (Yen et al., 2005), cardioprotective (Raphael et al., 2004), neuroprotective (Lomb et al., 2009), antimicrobial (MeRkl et al., 2010), anti-inflammatory and myoprotective activity (Philipp et al., 2006). Therefore, in the present study, it was hypothesized that prolyl hydroxylase inhibitor EDHB may facilitate in improving physical performance in rats by stabilizing HIF-1.

Most of the studies on hypoxia mimetics have focused on ischemia/reperfusion injury and there is scarcity of data on their efficacy in improvement of physical performance. Therefore, to understand the effectiveness of hypoxia adaptation in exercise physiology, the study was undertaken to assess the modulatary role of prolyl hydroxylase inhibitor EDHB as a mediator of hypoxic preconditioning by stabilizing HIF-1, thus, improving physical performance.

Aim:

The aim of the study was to explore the potential of prolyl hydroxylase inhibitor as preconditioning agent for stabilizing HIF and facilitating improvement in physical performance

Objectives:

The following objectives were proposed:

- To screen different 2-oxoglutarate analogues (PHD inhibitors) by cell viability studies.
- To study the cytoprotective/ antioxidative efficacy of selected candidate (EDHB): *in-vitro* studies.
- Dose response and dose optimization studies to evaluate the efficacy of EDHB in improving physical performance using rat as an animal model.
- To investigate the role of EDHB in the activation of the cellular oxygen sensing system
- To assess protective effectiveness of EDHB against exercise induced oxidative damage.
- To explore its usefulness in regulating cellular energy metabolism.
- To determine the efficacy of EDHB in promoting mitochondrial biogenesis and myogenesis.
- EDHB efficiency to confer protection against muscle damage by histopathological studies.

➢ Screening of 2-oxoglutarate analogues (PHD inhibitors) and cytoprotective/ antioxidative efficacy of EDHB: *In vitro* studies.

Methodology:

L6 (Rat muscle myoblast cells) cells were exposed to different levels of hypoxia (0.5, 1.0, 3.0 $\%O_2$) to optimize the hypoxic exposure conditions by cell viability assay. PHD inhibitors viz, Dimethyl Oxalyl Glycine, Ethyl 3, 4 Diethoxy Benzoate, Ethyl 3, 4 Dihydroxy Benzoate were screened for their cytoprotective efficacy by MTT assay. EDHB was found to be most potent in improving cell viability under hypoxia and thus used in further studies. The cells were then exposed to optimum hypoxia (0.5% O_2) for different time durations (12, 24, 48 hrs) after preconditioning with EDHB. After hypoxia exposure, level of HIF-1α was estimated by ELISA and oxidative stress markers were measured viz., ROS generation by determination of highly fluoresecent compound 2', 7'-dichlorfluorescein (DCF), Protein oxidation by estimating carbonyl groups after derivitization of proteins with dinitrophenyl hydrazine (DNPH) and lipid peroxidation by determination of malondialdehyde levels. Antioxidant status was evaluated by estimating levels of GSH (Kum-Tatt and Tan, 1974), Superoxide dismutase (SOD) (Kakkar et al., 1984), Glutathione peroxidise (GPx) was determined using commercially available kits (Randox, UK) as per manufacturer's instructions.

Salient findings:

No significant fall in cell viability was observed at 3% O_2 after 72 hr while there was a little decrease in viability at 1% O_2 after 48 and 72 hr. Exposure to 0.5% O_2 led to reduction in cell viability from 81% at 24 hr to 60% after 72hr exposure. Balance between oxidant and antioxidant levels determines the extent of protection of cells from oxidative stress. There was enhanced cellular viability and augmentation in the levels of HIF-1α. Reduced levels of protein oxidation and malondialdehyde indicate decrease in oxidative stress on exposure to hypoxia. EDHB treatment also conferred superior anti-oxidant status as there was boost in the levels of GSH and antioxidant enzymes like SOD and GPx. Thus the improved antioxidant status, increased expression of MT-1 and HIF-1 mediated induction of HO-1 might be responsible for according protection against oxidative damage, thereby improving the adaptive responses under hypoxic conditions, thus emphasizing the significant cytoprotective role of EDHB in protecting against oxidative stress under hypoxia. These results thus emphasize the potential of EDHB as cytoprotective agent against hypoxia induced oxidative damage

➢ Dose response and dose optimization studies with EDHB: In vivo studies

Methodology:

Animals:

All animal procedures were approved by the Institutional Animal Ethic Committee and were in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animal, India (CPCSEA). Efforts were made to minimize animal suffering and number of animals used for experimental purpose. Male Sprague-Dawley rats (170 ± 10 g) were used for the study. Animals were maintained in the institute animal house facility at (24±2°C) with 12-h light/dark cycle, relative humidity was maintained at 40-50%. Animals were fed standard pelletized diet (Lipton India Ltd.) and water ad libitum. Body weight, food and water intake were measured daily. Animals were maintained under the surveillance of a qualified veterinarian from institute

Dose optimization:

Rats (n=8/group) were subjected to treadmill training for 10 days with belt speed 6 m/min for 20min. The time and speed were increased 5 min/day and 1m/min until the speed reached 15m/min for 50 min. EDHB at the dose ranging from 25-150mg/kg bw (i.p) was supplemented to rats during the last 3 days of training. On 11th day, running time till exhaustion was measured at belt speed of 24m/min with 5% inclination. From the results of above experiment, the minimal effective dose was found to be 50 mg/kgbw. Therefore, further experiments were carried out at this concentration.

In another set of experiment, animals were divided into 4 groups (n=8/ group). (1) Control sedentary (CS) (2) EDHB (50mg/kg bw) treated sedentary (DS) (3) control training

(CT) (4) EDHB treated training (DT). The rats in treated groups were supplemented with optimum dose of 50 mg/kg bw EDHB (i.p.) for three days while control group was supplemented with vehicle (5% DMSO). The training protocol in the training groups was same as mentioned above. On 11th day, exhaustion time was measured. Blood was collected by retro-orbital puncture. Animals were sacrificed under anaesthesia and red gastrocnemius muscle was separated and stored at -80^{0}C for further studies.

Salient findings:

There was 2.4 times increase in running time till exhaustion in the animals given training only (CT) (81.3 ± 9.5 min) as compared to control sedentary (CS) (34 ± 5.8 min). Supplementation of different doses of EDHB (25, 50 and 100 mg/kg bw) along with training further increased exhaustion time (107± 7.4, 130.3 ± 6.4 and 140.5 ± 5.5 min respectively) and significantly ($p<0.01$) as compared to control trained rats. Maximum increase was observed at 50 and 100 mg/kg bw EDHB and effect was almost same in both these groups. Therefore, 50 mg/kg bw EDHB was used in all the further studies. There was augmentation in the physical performance in rats supplemented with optimum dose of EDHB (50 mg/kg bw). The running time till exhaustion was found to be 1.5 times higher in EDHB trained rats as compared to control trained rats.

➢ Role of EDHB in activation of cellular oxygen sensing system

Methodology:

Blood was collected by retro orbital puncture into heparinised tubes as mentioned above. Haemoglobin (Hb) and Haematocrit (Hct) were analysed in heparinised blood by blood cell counter (MS-4, Desing Laboratories, France). Parameters to be estimated in the blood were determined immediately. Remaining blood was centrifuged at 3000 rpm for 20 min and the plasma was processed immediately. The whole gastrocnemius muscle was collected from the calf region of hind limbs. The red gastrocnemius muscle was then separated from the whole muscle for further analysis. Expression of HIF-1α, Vascular Endothelial Growth Factor (VEGF), Erythropoetin (EPO) and PHDs was studied in muscle by western blotting and ELISA. Protein expression of myoglobin was also studied by western blotting. Transcriptional studies of hypoxia signalling pathway were done by isolating mRNA by Trizol method and then cDNA was prepared followed by PCR array as per as per manufacturer's protocol (Qiagen, USA).

Salient findings:

There was significant increase in hematocrit and blood haemoglobin level in supplemented rats as compared to control. EDHB supplementation led to increase in the levels of HIF-1α as estimated by ELISA and confirmed by western blotting suggesting stabilization of HIF-1α leading to elevated levels of VEGF and Epo, indicating improved angiogenesis and blood oxygen carrying capacity. Further increase was observed in levels of these proteins in EDHB supplemented rats along with training as compared to trained control rats. Prolyl hydroxylase domain (PHD) protein is involved in the oxygen regulated

hydroxylation of proline residues of HIF under normoxic condition leading to degradation of HIF. Chemical preconditioning with PHD inhibitor EDHB can lead to stabilization of HIF under normoxic condition. A marked decrease was observed in the expression of PHD1 and PHD2 with EDHB supplementation as compared to control ($p<0.01$) resulting in enhanced expression of HIF-1α. PCR array studies revealed upregulation of various hypoxia regulated genes i.e., HIF-1α, VEGF, Epo, myoglobin (Mb), hexokinase, hemeoxygenase-1, lactate dehydrogenase, Phosphofructokinase, MCT 4, GLUT-1, PDK1, pyruvate kinase, Glycogen synthase in the EDHB supplemented group as compared to control.

- **Antioxidant and anti-inflammatory response of EDHB against exercise induced damage**

Methodology:

Antioxidant status was estimated by measuring the levels of Reduced glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidise (GPx) and Glutathione –S-transferase (GST). GSH was measured in blood and muscle by the method of Kum Tatt (Kum-Tatt and Tan, 1974). The rate of formation of TNB was followed at 412 nm. GSSG levels in muscles were measured fluorimetrically by the method of Hissin &Hilf (Hissin and Hilf, 1976). Fluorescence was measured at an excitation wavelength of 350 nm and emission at 420 nm. Activity of GPx was determined using commercially available kits (Randox, UK) as per manufacturer's instructions. GST was determined using protocol described by Habig et al.(Habig et al., 1974) and optical density was recorded at 340 nm. SOD was determined using protocol described by Kakkar et al. (Kakkar et al., 1984). Absorbance was measured at 540 nm at an interval of 15 sec for 180 sec. A single unit of enzyme was expressed as, 50% inhibition of NBT (Nitroblue tetrazolium) reduction/min/mg protein.

Oxidative stress was estimated by measuring the levels ROS, MDA and protein oxidation. MDA level was assayed by using 2-thiobarbituric acid reagent using 1,1,3,3 – tetraethoxypropane as standard. ROS generation was estimated by DCFH-DA method via determination of highly fluoresecent compound 2', 7'- dichlorfluorescein (DCF). Protein oxidation was measured by determining the carbonyl groups after derivitization of proteins with dinitrophenyl hydrazine (DNPH). The levels of various proinflammatory cytokines viz., IFN-γ (interferon – γ), TNF- α (tumor necrosis factor – α), MCP-1 (monocyte chemoattractantprotein-1) and anti-inflammatory cytokines, IL-6 (Interleukin-6), TGF-β (transforming growth factor-β) and IL-10 (interleukin-10) were estimated in plasma by ELISA (Opti-Eia kits) as per manufacturer's instruction. (BD Bioscience, CA). The expression profile of various proteins viz., Hemeoxygenase-1(HO-1), Metallothionein (MT-1), Nuclear Factor Erythroid 2-Related Factor (Nrf2), Nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB), and cyclooxygenase 2 (Cox-2) was determined by immunoblotting using specific antibodies.

Salient findings:

Studies have shown that strenuous exercise leads to free radical formation and lipid peroxidation in skeletal muscle and erythrocytes. The rise in free radical concentrations could exceed the protective capacity of cell antioxidant defense systems (Duthie et al., 1990). Exhaustive exercise in rats leads to the depletion of GSH levels and the training through treadmill running was demonstrated to increase oxidative stress in liver, muscle and blood (Alessio and Goldfarb, 1988). Our study showed significant improvement in antioxidant status as observed by increase in GSH, SOD, GPx, GST and decrease in MDA, ROS and protein oxidation. Enhanced expression of anti-oxidative proteins HO-1, MT-1, Nrf2, also contributed in decreasing training induced oxidative stress in rats. There was significant decrease in the protein expression of inflammatory proteins viz., NF κB, TNFα and Cox-2 in EDHB treated groups as compared to controls. NF κB is a key transcription factor that regulates inflammatory mediators. TNFα and Cox2 are also modulators of inflammation and decrease in the expression of these indicates protection to the muscle against inflammation. Increased expression of NF κB, TNFα and Cox-2 is associated with inflammation therefore levels of circulatory pro and anti-inflammatory cytokines in plasma confirmed the anti-inflammatory effect of EDHB. There was significant decrease in the levels of proinflammatory cytokines IFN-γ, TNF- α, MCP-1 while increase in anti-inflammatory cytokines IL-6, TGF-β, IL-10 in the EDHB supplemented rats as compared to control revealed anti-inflammatory property of the EDHB. All these findings suggest the potential of EDHB as therapeutic agent for improving endurance performance by facilitating hypoxia adaptation in the skeletal muscle and ameliorating oxidative stress and inflammation.

➢ Effect of EDHB in improving cellular energy metabolism

Methodology:

Activities of enzymes involved in cellular respiration in red gastrocnemius muscle were measured spectrophotometrically. Citrate synthase (CS), Succinate dehydrogenase (SDH), Hexokinase, Phosphofructokinase (PFK) were determined in muscle by the methods of (Srere, 1963;Veeger et al., 1969;Ling et al., 1966) respectively. Lactate dehydrogenase activity (LDH), lactate and glucose level were determined using commercially available kits (Randox Laboratories Ltd, Crumlin, UK) as per the manufacturer's instructions. ATP, pyruvate, glycogen and acetyl CoA levels were measured by using commercially available kits (Sigma-Aldrich, St Louis, MO, USA) as per manufacturer's protocol. Protein expression studies of glucose transporters, GLUT-1 & 4, Pyruvate dehydrogenase kinase (PDK1 & 4), Carnitine palmitoyl transferase-1 (CPT1) and lactate transporters were studied in muscle by western blotting.

Salient findings:

Endurance exercise such as prolonged running induces an increase in muscle respiratory capacity (Booth and Baldwin, 1996). This adaptation includes increases in components of the mitochondrial respiratory chain, ATP Synthase, enzymes of the citrate cycle and enzymes involved in fatty acid (Oscai and Holloszy, 1971;Chi et al., 1983). This enhancement of muscle respiratory capacity plays a major role in improvement in exercise performance and

endurance induced by training (Holloszy and Coyle, 1984). EDHB augments metabolic status in skeletal muscle by upregulation of HIF mediated Glut1 and Glut 4, thus enhancing glucose uptake. Blood glucose and muscle glycogen are essential for prolonged strenuous exercise, and exhaustion can result either from development of hypoglycemia or depletion of muscle glycogen. Enhanced glucose uptake may lead to active glycolysis as there was enhanced activity of hexokinase and PFKm. The adaptations induced by endurance exercise training result in a marked sparing of carbohydrate during exercise, with an increased proportion of the energy being provided by fat oxidation. PDK1 and 4 suppresses the pyruvate dehydrogenase complex and thus conversion of pyruvate to acetyl CoA. We observed upregulation of HIF regulated PDK1 and 4 expressions pointing towards decrease in acetyl CoA. But the enhanced level of acetyl CoA as observed in this study in EDHB supplemented groups may be due to enhanced fatty acid oxidation as there was rise in the expression of CPT-1 and peroxisome proliferator activated receptor (PPAR)-α after EDHB supplementation which helps in fatty acid oxidation. Thus the enhanced level of Acetyl CoA resulted in augmentation of Kreb cycle which was evident by increase in SDH and CS resulting in production of high levels of ATP. Membrane bound monocarboxylate transporters (MCT) facilitate cell to cell exchange and two isoforms MCT-1 and MCT-4 are the most active forms in skeletal muscles. MCT-1 channels the lactate from blood to skeletal muscle and MCT-4 is involved in cellular lactate extrusion (Morris and Felmlee, 2008). There was increase in muscle LDH activity and decrease in muscle pyruvate levels suggesting conversion of pyruvate to lactate. Further reduced expression of MCT1 and increase in MCT4 after EDHB supplementation suggest transport of lactate from muscle to blood. But there was decrease in plasma lactate which might be due to enhanced transport of lactate from blood to liver and conversion to pyruvate as there was rise in liver pyruvate levels. Pyruvate is utilized in gluconeogenesis leading to increased glycogen levels in liver as observed in EDHB supplemented rats as compared to control rats. These results suggest boost in physical performance through escalation of aerobic respiration.

➢ Efficacy of EDHB in promoting mitochondrial biogenesis and myogenesis

Methodology:

To study the effect of EDHB on mitochondrial biogenesis and myogenesis, expression studies were done at transcription and translational levels. Proteins expression studies of mitochondrial transcription factor A (mtTFA), peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α), PPAR-α, PPAR-β, Estrogens related receptor (ERR-α), Muscle atrophy F- box (MAFbx), Myocyte enhance factor (MEF-2), myogenic factor (Myf) 6, Myf5, Myogenin, MyoD, nuclear respiratory factor (NRF-1) were done by western blotting as mentioned above. Transcriptional studies were done by isolating mRNA by Trizol method and then cDNA was prepared followed by PCR array as per as per manufacturer's protocol (Qiagen, USA).

Salient Findings:

PCR array studies revealed upregulation of various genes of myogenesis which includes genes of energy metabolism viz., CS, HK2, PDK4, Glut4, genes of myogenesis i.e., MEF2, Myf5, Myf6, Myod1, Myog, PGC-1, Mb. The PGC-1α has recently been identified as a nuclear factor critical for coordinating the activation of genes required for mitochondrial biogenesis in cell culture and rodent skeletal muscle (Wu et al, 1999). PGC-1α does not bind to DNA itself rather interacts with selected transcription factors already bound to the promoter region of the target genes. It has been found to coactivate NRF-1, PPAR-γ and PPAR-α; to increase NRF-1 and NRF-2 gene expression and to stimulate mitochondrial biogenesis (Wu et al., 1999). Expression studies have shown that EDHB supplementation resulted in increased expression of various exercise responsive proteins. Increased expression of mtTFA, PGC-1α, NRF-1 and PPAR-α was observed as mitochondrial adaptation to endurance training in humans is associated with activation of PGC-1α, as well as its downstream transcription factors (NRF-1 and 2, mTFA) and CS which induce coordinated expression of mitochondrial transcripts. Along with these there was increase in the expression of myogenic genes including UCP3, ERRα, MEF2, Myf6, Myf5 and MyoD. Enhanced expression of all these genes following EDHB supplementation suggests boost in mitochondrial density and myogenesis in muscles to sustain from fatigue for a longer period and thus protects muscle from exercise induced damage.

➢ Histopathological studies

Methodology:

In another set of experiment (n=3), after exhaustion time measurement, animals were anaesthetized as above and were immediately perfused with ice cold PBS followed by fixation in 4% para-formaldehyde (dissolved in 0.1M PBS pH 7.4). Red gastrocnemius muscle was removed carefully, fixed in same fixative for 24hr at room temperature, dehydrated through graded alcohol and embedded in paraffin. Approximately 5µm sections were made and stained with haematoxylin and eosin according to standard procedures for morphological analysis. The sections were analysed under Labcom (Germany), trinocular research microscope at 40x. The photomicrographs were captured by Canon (Tokyo, Japan) digital camera attached to light microscope.

Salient findings:

Histopathology studies revealed myoprotective effect of EDHB in supplemented rats as control sedentary group showed greatest degree of muscle damage with rounding and reduction in fibre diameter whereas EDHB training group showed improvement with no rounding or reduction in fibre diameter.

Summary:

EDHB supplementation leads to increase in aerobic metabolism in skeletal muscles and also protect muscle from free radical induced damage by activating antioxidant system and various antioxidant enzyme activities. Increased expression of HIF regulated genes (i.e. Epo, HO-1, VEGF, MT-1) confirms activation of oxygen sensing system in skeletal muscle

that leads to hypoxia adaptation. Significant elevated levels of exercise responsive and myogenic genes (viz. UCP3, ERRα, MEF2, Myf6, Myf5 and MyoD) and metabolic enzyme activities (CS, SDH, PFK, HK) suggest boost in mitochondrial density and myogenesis in muscles to sustain from fatigue for a longer period and thus protects muscle from exercise induced damage. Therefore, in conclusion EDHB supplementation enhances physical performance in rats by switching on several intricate molecular pathways and protects muscle from exercise induced damage.